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Plant Immunity: AvrPto Targets the Frontline

Bacterial pathogens must suppress host defences to cause disease. New research shows that the *Pseudomonas* effector protein AvrPto does so by directly targeting plant transmembrane receptor kinases involved in bacterial perception.

Cyril Zipfel and John P. Rathjen

An old saw in plant pathology states that most plants are resistant to most pathogens. An important aspect of this phenomenon is host recognition of immutable pathogen molecules, known as PAMPs (for pathogen-associated molecular patterns), by pattern recognition receptors (PRRs). Only a few plant PRRs are known; the plasma-membrane-localised leucine-rich-repeat receptor kinases (LRR-RK) FLS2 and EFR recognise the bacterial PAMPs flagellin and EF-Tu, or their peptide epitopes flg22 and elf18, respectively [1]. If PAMP recognition is not evaded or suppressed, host immunity is elicited and pathogen growth is halted. Importantly, Zhou and colleagues [2], in a recent issue of *Current Biology*, now show that the bacterial virulence factor AvrPto targets PRRs directly to suppress PAMP recognition in host plants.

Bacterial pathogens secrete a suite of virulence 'effector' proteins through a specialised type III secretion system (TTSS) [3]. The model pathogen, *Pseudomonas syringae* pv *tomato* DC3000 (*Pto* DC3000), secretes more than 30 effectors, and mutants defective in the TTSS machinery ('*ttss*' mutants) are not infectious. However, mutants lacking individual effector genes display subtle or no virulence phenotypes, suggesting that effectors act redundantly or additively. Nevertheless, several effectors have been shown to inhibit or suppress plant immune responses and to contribute to virulence [3,4]. Despite these advances, in most cases the effectors' targets in the plant cell are still unknown, reflecting our generally poor knowledge of plant immune-signalling pathways.

Plant immunity comprises several layers of recognition of which PAMP-triggered immunity (PTI) is the first. A secondary layer involves recognition of effectors by dedicated resistance (R) proteins. To infect a given host, microbes must either avoid PTI or suppress it through the actions of effectors. In turn, some plants have evolved resistance (R) proteins to detect these effectors, causing effector-triggered immunity (ETI), which is often accompanied by a form of cell death known as the hypersensitive response (HR). The dynamic interplay of these two forms of immunity as the host struggles to recognise elusive pathogens reflects the evolutionary pressures of these intimate relationships [5,6].

The *Pto* DC3000 effector protein AvrPto is a small triple-helix protein that, like several other effectors, is targeted to the plasma membrane by N-myristoylation [7]. Although AvrPto contributes demonstrably to pathogen virulence, it was identified initially by its ability to induce ETI in tomato plants carrying an effector recognition complex composed of the protein kinase Pto and Prf, a canonical R protein of the nucleotide binding-LRR family [6] (Figure 1). AvrPto interacts directly with Pto in tomato cells. However, AvrPto contributes to virulence in tomato lines lacking Pto and/or Prf [8–10]. Strikingly, ectopic overexpression of AvrPto in the plant model *Arabidopsis thaliana* restores growth of a *Pto* DC3000 *ttss* mutant to almost wild-type levels [11], suggesting that AvrPto suppressed PTI to a sufficient level to allow growth of this strain. Moreover, transgenic expression of AvrPto suppressed the expression of genes encoding defence and secreted cell-wall proteins and also inhibited callose deposition induced by a *Pto* DC3000 *ttss* mutant. AvrPto was therefore proposed to suppress cell-wall-based plant defences. However, several subsequent publications reported that AvrPto seems to work very early in PTI, because AvrPto expression in *Arabidopsis* and *Nicotiana benthamiana* inhibits several early markers of PTI [12–14]. Interestingly, AvrPto expression inhibits early responses induced by several PAMPs [13,14]. Taken together, these results showed that AvrPto must target signalling components directly at, or immediately proximal to, recognition events at the plasma membrane. The major question, however, remained; how does it do it?

Until recently, no biochemical function could be assigned to AvrPto. Recent structural work suggests that AvrPto acts as an inhibitor of Pto by occluding the kinase catalytic cleft [15]. Somewhat confusingly, the kinase-inhibition activity of AvrPto is dispensable for elicitation of Pto–Prf-mediated resistance [15], suggesting that an alternative protein kinase target(s) might underlie the virulence activity of AvrPto. Based on homologies between the kinase domain of Pto and those of FLS2 and EFR, Zhou and colleagues [2] postulated that AvrPto might interact with and inhibit these LRR-RLKs. Indeed, AvrPto interacts with FLS2 and EFR both *in vitro* and *in vivo* when expressed ectopically in plant cells. Furthermore, AvrPto inhibits autophosphorylation of FLS2 and EFR in a dose-dependent manner. Thus, AvrPto is an inhibitor of PRR kinase domains (Figure 1), consistent with its plasma-membrane localisation and variety of suppression activities.

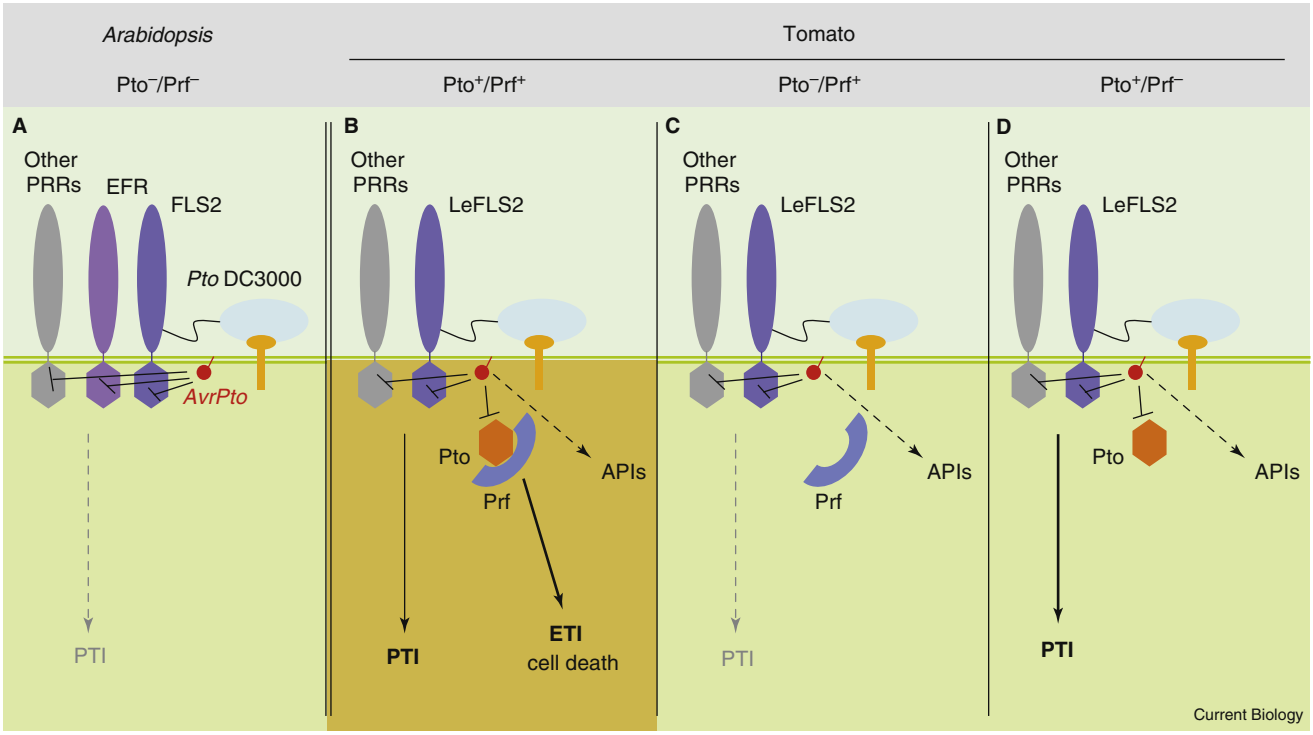

Figure 1. AvrPto suppresses PTI in *Arabidopsis* and tomato.

(A) *Pseudomonas syringae* pv *tomato* DC3000 (*Pto* DC3000) binds to cell walls and secretes multiple type III effectors, including AvrPto, into the cytoplasm. AvrPto binds PRRs and inhibits their kinase activity, leading to PTI suppression. (B) In tomato containing the Pto–Prf effector recognition complex, Pto competes with PRRs for AvrPto binding, leading to elicitation of cell death through Prf-mediated ETI. (C) In the absence of Pto, AvrPto inhibits PTI through PRRs and potentially other AvrPto-interacting proteins (APIs). (D) In the absence of Prf, AvrPto is predicted to be sequestered by Pto without induction of ETI, leading to ineffective PTI suppression.

While it is clear that AvrPto has the potential to interact with and inhibit specific LRR-RLKs, it has yet to be shown directly that these interactions occur during an actual infection. One technical barrier that prevents the demonstration of such interactions is that TTSS delivers vanishingly small amounts of the effector to each cell. However, a model in which AvrPto acts as a kinase inhibitor must take into account the concentration of the delivered effector. While the concentration of AvrPto within the infected cell is unknown, it is possible that AvrPto and other N-myristoylated effector proteins exist in membrane microdomains of high local concentration. Analysis of AvrPto and FLS2 mutants provides supporting evidence that these proteins interact *in vivo*. Given the similarities between the kinase domains of Pto, FLS2 and EFR, it was tempting to speculate that the mechanisms involved in their interactions with AvrPto would be similar. Some, but not all, AvrPto residues that specify its interaction with Pto are also required to suppress PTI in *Arabidopsis* [13]. Similarly, Xiang *et al.* [2] show that some mutations in the GINP loop of AvrPto that abolish the interaction with Pto also reduce the interaction with the kinase domains of the receptor kinases and reduce the inhibition of these kinases. Mutations in the Pto ATP-binding site abolish the AvrPto–Pto interaction, and homologous mutations in FLS2 also compromised the AvrPto–FLS2 interaction *in vitro* and *in vivo*. However, it is premature to draw too many conclusions here about the mechanism of AvrPto action, and a more precise description will require in-depth structural work.

Do the current data explain all of the virulence activity of AvrPto? FLS2 (or a member of this pathway) is clearly an important target, because growth of bacteria lacking AvrPto is reduced on wild-type hosts, but recovers when a receptor kinase is absent [2]. While it is clear that AvrPto retains some target specificity — for example, it did not interact with PKS3, a kinase with roles in abiotic stress responses — it is possible that different AvrPto targets assume more importance in certain host–pathogen contexts. However, the kinase domains of all ~600 plant receptor kinases are monophyletic [16], so it seems likely that other members will also be targeted by AvrPto. These potential additional targets could explain the startling suppression of PTI by ectopic overexpression of *AvrPto* in *Arabidopsis*, leading to growth of the *Pto* DC3000 *ttss* mutant. With this in mind, it is interesting that an *Arabidopsis* receptor kinase of unknown function, At2g23200 [2], interacts with and is inhibited by AvrPto *in vitro*. The function of this kinase in plant immunity needs further investigation. Are there other possible targets? BAK1/SERK3 is a receptor kinase with general roles in PTI that dimerises with FLS2 (and probably other PRRs) immediately after elicitation [17,18]. Inhibition of BAK1 would suppress multiple pathways by targeting a single common member. BAK1 is not required for the AvrPto–FLS2 interaction [2], but AvrPto might still bind BAK1 and/or disrupt the interaction of FLS2 and other PRRs

with BAK1. Finally, two intriguing AvrPto-interacting proteins, Api2 and Api3, are putative small GTPases with homologies to human Rab8 and yeast Sec4p proteins [19]. However, the function of Api proteins in PTI and the relevance of their interaction with AvrPto for the promotion of virulence have never been assessed.

Certain results from this and previous studies have prompted a re-evaluation of current models for effector recognition during ETI. Recognition of effectors by R proteins can be direct or indirect. In the case of indirect recognition, the dominant hypothesis suggests that R proteins monitor cognate host proteins, designated 'guardees', for modifications induced by effectors as part of a virulence strategy [6]. However, neither Pto or Prf seem to be plausible virulence targets. Current and previous work suggests that there may be competition between Pto and FLS2 for AvrPto binding [2,13]. This forms the basis of a new model for effector recognition, in which Pto acts as a 'decoy' for FLS2 (Figure 1). In this model, Pto is a mimic of the FLS2 kinase domain, but elicits strong defences through ETI. In the case of AvrPto, a perfect test of the model can be carried out by examining FLS2 inhibition by AvrPto in tomato lines that contain Pto but lack Prf (Figure 1). Overall, the current work, together with a previous study on viral effectors [20], shows an important new strategy for effector function that is likely to be general for all plant–microbe interactions.

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Trans-Synaptic Plasticity: Presynaptic Initiation, Postsynaptic Memory

A novel mechanism of persistent facilitation induced by serotonin at *Aplysia* synapses depends upon rapid postsynaptic protein synthesis and increased responsiveness to glutamate; whereas the memory for this synaptic change is postsynaptic, the initiating signal may be an increase in spontaneous release of glutamate from the presynaptic terminals.

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The analysis of cellular and molecular mechanisms of synaptic plasticity that contribute to learning has long been organized around simple dichotomies, such as presynaptic *versus* postsynaptic mechanisms. While these can be useful distinctions for guiding experimental analysis, the underlying biology may be more complex, as a diversity of mechanisms contributes to plasticity at individual synapses. According to the commonly held perspective, long-term potentiation in the CA1 region of hippocampus involves strictly postsynaptic modulatory mechanisms [1], whereas in the marine mollusc *Aplysia*, facilitation at synapses between sensory neurons and motor neurons involves presynaptic mechanisms [2]. Recent work from two laboratories [3,4] on these sensorimotor synapses now suggests a novel form of trans-synaptic plasticity, in which the presynaptic neuron plays an important initiating role, while persistent changes in the postsynaptic cell underlie the stable increase in synaptic strength. The coupling between presynaptic and postsynaptic cells appears to be mediated by an increase in spontaneous vesicle release, a phenomenon previously thought to have no signaling value. While we focus here on facilitation at the *Aplysia* sensorimotor synapse, the